Defining Field Cancerization of the Skin Using Noninvasive Optical Coherence Tomography Imaging to Detect and Monitor Actinic Keratosis in Ingenol Mebutate 0.015%–Treated Patients

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ABSTRACT

Objective: The objective of this study was to assess the ability of optical coherence tomography to detect clinical and subclinical actinic keratoses confirmed by histopathology. The efficacy of ingenol mebutate treatment of actinic keratosis was also evaluated using optical coherence tomography, and correlation of treatment efficacy with severity of local skin reactions was determined. **Design:** Single-arm, open-label, split-face study. **Setting:** Hospital outpatient clinic. **Participants:** Male subjects (N=30) with seven actinic keratoses. **Measurements:** A suspected actinic keratosis and the normal-appearing, perilesional skin were imaged, biopsied for histopathologic analysis, and the results compared with the clinical and a blinded optical coherence tomography diagnosis. Treatment with ingenol mebutate gel 0.015% was randomly administered to three clinically suspected actinic keratoses and the perilesional skin; three additional, suspected actinic keratoses lesions and perilesional areas were left untreated. Clinical and optical coherence tomography images were obtained for all lesions. Severity of local skin reactions was recorded to evaluate the relationship between local skin reaction and treatment effect. **Results:** Optical coherence tomography analysis had a 100-percent (28/28) correlation with the clinical diagnosis of actinic keratosis and detected 16 of 22 (73%) histopathologically confirmed subclinical lesions from perilesional skin sites. By optical coherence tomography assessment, the clearance rate for clinically observed lesions was 76 percent for ingenol mebutate-treated areas versus 11 percent for untreated areas; the clearance rate for treated subclinical lesions was 88 percent versus 43 percent for untreated areas. Clearance rates did not vary with the severity of the local response. **Conclusion:** Optical coherence tomography is effective at detecting clinical and subclinical actinic keratoses and monitoring their response to treatment. (*J Clin Aesthet Dermatol.* 2016;9(5):18–25.)

Long-term, chronic exposure to ultraviolet radiation from sunlight can induce DNA damage in epidermal keratinocytes and promote the formation of skin abnormalities, including actinic keratosis (AK).[1–3] AKs present as skin-colored to reddish-brown macules, papules, or plaques with superficial gritty scale. Gene expression patterns and histopathologic analysis suggest that AKs are part of a disease continuum, starting with subclinically sun-damaged skin and ultimately leading to the potential development of squamous cell carcinoma (SCC).[4–6] Over time, AKs may resolve, remain stable, or progress. The risk for progression to invasive SCC over a 10-year period for patients with multiple AKs has been estimated at 10 percent.[7]

A primary challenge in the management of AKs is the possibility of field cancerization, a term that describes a

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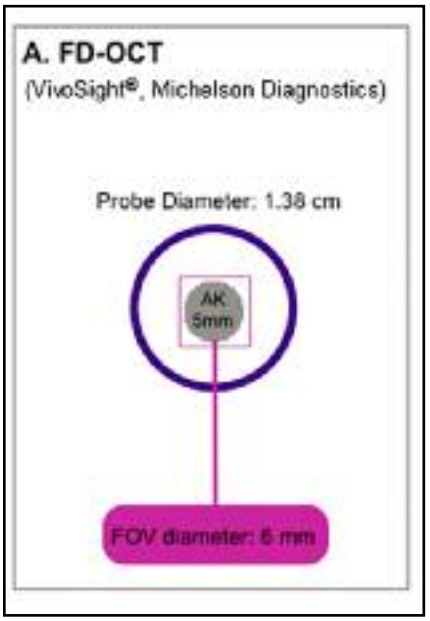


Figure 1. Probe size and field of view (FOV) for a commercially available OCT device. Probe size and FOV in the context of an AK (grey circle) for Fourier domain OCT (FD-OCT) is shown (A). The probe that contacts the skin is circular, whereas the imaged FOV is a square grid. The relatively low probe:FOV ratio and the ample 6mm FOV diameter of the FD-OCT are well-suited to imaging AKs in the clinic.

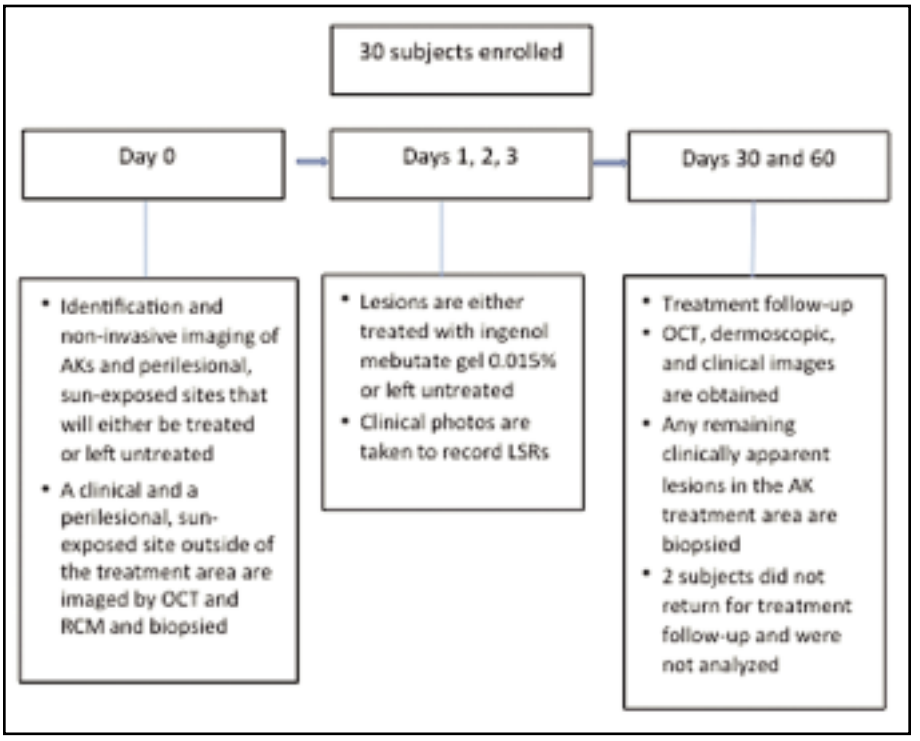


Figure 2. Study methods and design

biologic process in which large areas of cells are affected by a carcinogenic alteration resulting from a prolonged period of exposure to an injurious environment. The concept of field cancerization, introduced by Slaughter et al in 1953 with reference to histologic analysis of oral cancers,[8] has been adapted to describe the damaging effects of ultraviolet light on the skin. Field cancerization presupposes an increased likelihood of future AKs and their progression to SCC. Determining and predicting which AKs may progress to SCC remains difficult, especially since visual identification of AKs is the current standard of care.[9] Various field-directed therapies are now available to treat not only clinically obvious lesions but also the underlying damage. It would thus be helpful to have validated, noninvasive techniques for visualizing, detecting, and monitoring the damaged field.

Optical coherence tomography (OCT) is one technology that offers considerable promise for noninvasive, real-time detection of AKs. OCT yields images of the tissue microarchitecture in both the horizontal and vertical planes down to a depth of approximately 2mm. Its resolution is only 5 to 10µm, however, which precludes the detection of distinct cellular features.[10,11] Nevertheless, the relatively large field of view (6mm) in OCT, and the low probe-to-field-of-view ratio (1.38cm:6mm) makes it well-suited to imaging broader areas of actinic damage (Figure 1). Noninvasive imaging devices show an inverse relationship between field-of-view size and cellular resolution. An ideal device for evaluating field cancerization is one that has just enough cellular resolution without compromising the scope of the field of view. Additionally, when using an imaging technique to monitor lesions at various intervals over time, it is important to minimize any discrepancy between the probe size and the imaging field. This is key to ensuring appropriate probe positioning over time. To date, OCT is used primarily in the research setting.

The aim of this pilot study was to further define actinic field cancerization of the skin by comparing OCT readings with conventional histopathology for the identification of clinical and subclinical AKs. The study also aimed to compare the results of clinical and OCT assessment in monitoring the effect of topical ingenol mebutate (Picato®) 0.015% gel[12] treatment of AKs on the face. A final objective was to investigate the relationship between the extent of lesion clearance and the severity of local skin reactions (LSRs) observed during ingenol mebutate treatment.

METHODS

This was a single-center, single-arm, open-label clinical study. The effects of ingenol mebutate gel 0.015%, applied once daily for three consecutive days in subjects with AKs of the face, were evaluated using clinical assessment and OCT imaging.

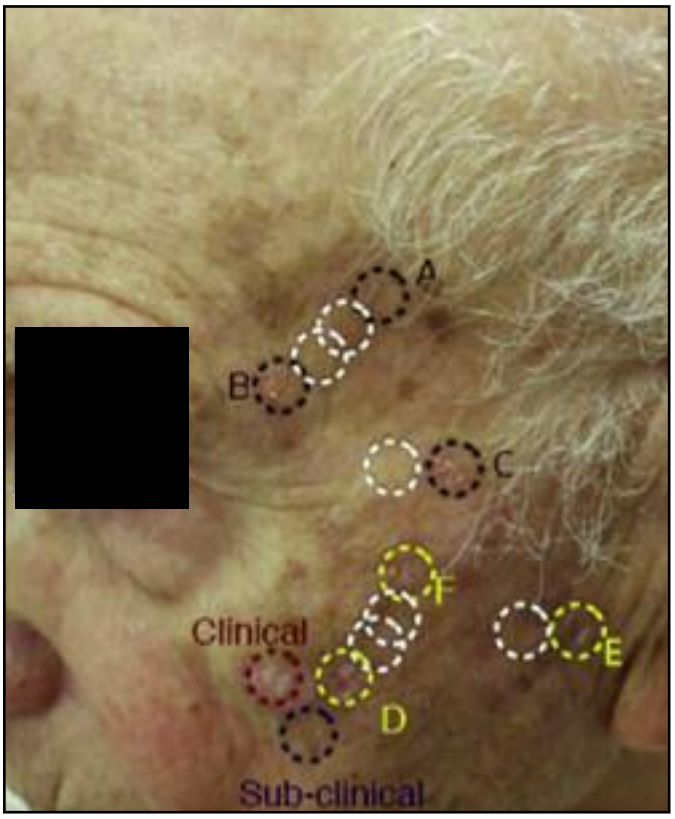


Figure 3. Assessment of AKs before and after ingenol mebutate gel 0.015% treatment. Discrete clinical (A–F) and adjacent, perilesional sites with keratinocyte atypia that might be sites of subclinical AKs (white circles) were identified. Regions in black circles (A–C) and nearby potential subclinical regions were treated with ingenol mebutate gel, while areas in the yellow circles (D–F) and their associated potential subclinical regions were left untreated. To evaluate the ability of OCT to identify AKs, a clinical (orange) and a subclinical (blue) lesion were imaged at baseline, and then were biopsied and assessed by conventional histopathology.

Subject selection. Subjects were male, ≥18 years of age, and were recruited from the Brooklyn Campus of the Veterans Affairs New York Harbor Healthcare System. Subjects were required to have at least seven clinically appearing AKs on the face in three separate areas: 1) a contiguous 25cm^2 area on the face containing three clinically typical, visible, and discrete AKs plus three perilesional, sun-exposed skin sites requiring AK treatment; 2) a comparable, 25cm^2 area containing three AKs and three perilesional sites on the opposite side of the face; and 3) one clinically visible AK and adjacent sun-exposed skin to be biopsied. Subjects were excluded if they had a history of, or evidence of, skin conditions other than AK and if there were lesions within the treatment area that had an atypical clinical appearance.

Recruitment and examination followed the path outlined in Figure 2. All participants provided written informed consent and allowed photographs of the selected areas to be taken.

AK treatment area. The treatment area for each subject was identified and recorded at baseline using a transparency sheet (Figure 3). Each treatment area consisted of a 25cm^2 contiguous area of the face that contained three clinical AKs as well as three perilesional areas that appeared as normal skin. A comparable area on the opposite side of the face served as an untreated area for each subject.

AK biopsy area. Outside of the treatment area, one clinical AK lesion and one adjacent sun-exposed area were selected at random to be imaged by OCT and then biopsied.

Subject treatment and assessment follow-up visits. Ingenol mebutate gel 0.015% was applied to the treatment area by the clinical research staff once daily for three consecutive days (Days 1, 2, and 3). Clinical photographs on each day of treatment were taken to evaluate LSRs. The efficacy of ingenol mebutate gel 0.015% was assessed at Day 60 by clinical assessment and by noninvasive OCT imaging. Any clinically apparent lesions remaining in the treatment area were biopsied.

Identification of lesions clinically. During the clinical examination, lesions were photographed with a standard digital camera (Canon X70, Sony Cyber-shot 14.4, or Nikon J1) and were inspected for clinical features consistent with AKs, which include papules, macules or plaques of variable thickness with scale, and surrounding erythema with a gritty, sandpaper-like texture.

Identification of lesions using OCT. OCT imaging was performed with a VivoSight® OCT Scanner (Michelson Diagnostics; Maidstone, Kent, United Kingdom). This is a multi-beam, Fourier domain (FD), swept-source OCT system (FD-OCT) that scans an area of 6mm × 6mm at a rate of 20k A-lines per second. Optical resolution is <7.5µm laterally and <5µm axially, with lesions scanned to a depth of approximately 2mm.

OCT scans were inspected for features consistent with AK. Specifically, the epidermal-dermal junction was analyzed for ill-defined borders, a haphazard pattern in the upper portions of the epidermis, evidence of scaling as indicated by hyperechogenic areas (white streaks), and epidermal thickening. These features are consistent with those reported in previous studies.[13–15] To be classified as an AK by OCT, a lesion was required to have an ill-defined dermoepidermal border, haphazard epidermal organization, and at least one other OCT indicator. To distinguish between AK and SCC, images were analyzed for the extent of epidermal thickening and haphazard patterning. The extent to which the criteria filled a scan was also considered, with smaller foci being more consistent with AK than with SCC (Figures 4A and 4B).

Evaluating LSR severity. The LSRs were evaluated for six parameters (erythema, scaling, crusting, pustulation, and erosion/ulceration) and graded on a scale from 0 to 3 (with higher numbers indicating greater severity), to a maximum score of 15. Subjects with a total reaction score from 0 to 3 were considered to have had a

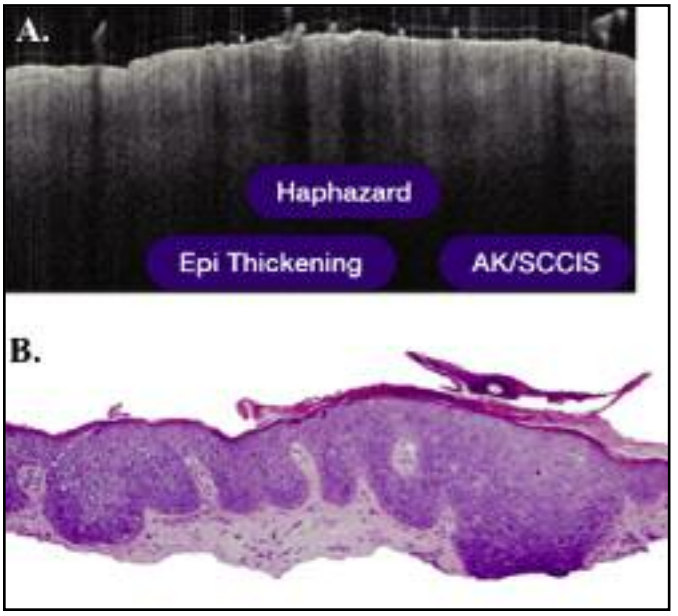


Figure 4. Detection of AKs by noninvasive imaging or histopathology. At baseline, subjects' lesions were imaged by OCT (A). The lesion was then biopsied and assessed using hematoxylin and eosin staining (B).
SCCIS=SCC *in situ*

TABLE 1. Summary of subject characteristics at baseline

CHARACTERISTIC	SUBJECTS (%)
Mean age [range] (years)	76 [67–93]
Race, Caucasian	100
Sex, male	100
Mean time since AK diagnosis (years)	7
Prior treatment for AK	100
Type of prior treatment	
Cryotherapy	89
Retin-A®/tretinoin	29
Fluorouracil	25
Photodynamic therapy	11
5-fluorouracil	4
Hydrophor™	4

mild reaction; subjects with a reaction score from 4 to 6 were considered to have a moderate reaction; and those with a reaction score of 7 and above were considered to have a severe reaction.

Analysis and statistical methods. ***OCT diagnostic accuracy.*** Noninvasive OCT imaging was used to examine one clinically identified AK and the perilesional, sun-exposed skin area on a site that was separate from the six sites that were either treated or untreated. The clinician's OCT diagnosis was compared with the clinical biopsy results to test for correlations.

Additionally, the baseline diagnoses of the treated and untreated lesions were established using both clinical assessment and OCT imaging. The baseline diagnoses for the clinical and subclinical lesions were compared.

Treatment effect. Analysis of treatment efficacy was carried out at the lesion level. If an individual's baseline lesions had become "normal" by Day 60, then we classified that lesion as "cleared"; otherwise, it was classified as "not cleared".

Relationship between treatment effect and LSR severity. Ingenol mebutate treatment causes LSRs. The level of reaction (mild, moderate, or severe) was recorded for each subject to determine whether the severity of the LSRs was related to the treatment effect.

All p-values were calculated using Chi-squared analysis. Confidence intervals (CIs) are expressed as 95% Wilson's CI, a method that performs well on proportions that tend toward the extremes.[16]

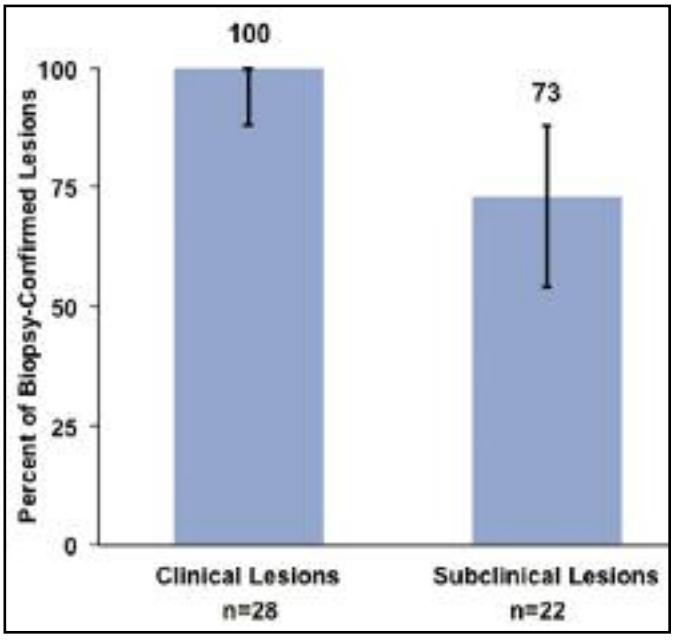


Figure 5. OCT in the identification of AKs. Lesions were imaged using noninvasive OCT and then biopsied. Histopathology revealed that 28 of 28 (100%) clinical lesions and 22 of 28 (79%) subclinical lesions were AK or SCC. Results are reported as a percentage of biopsy-confirmed lesions identified by OCT.

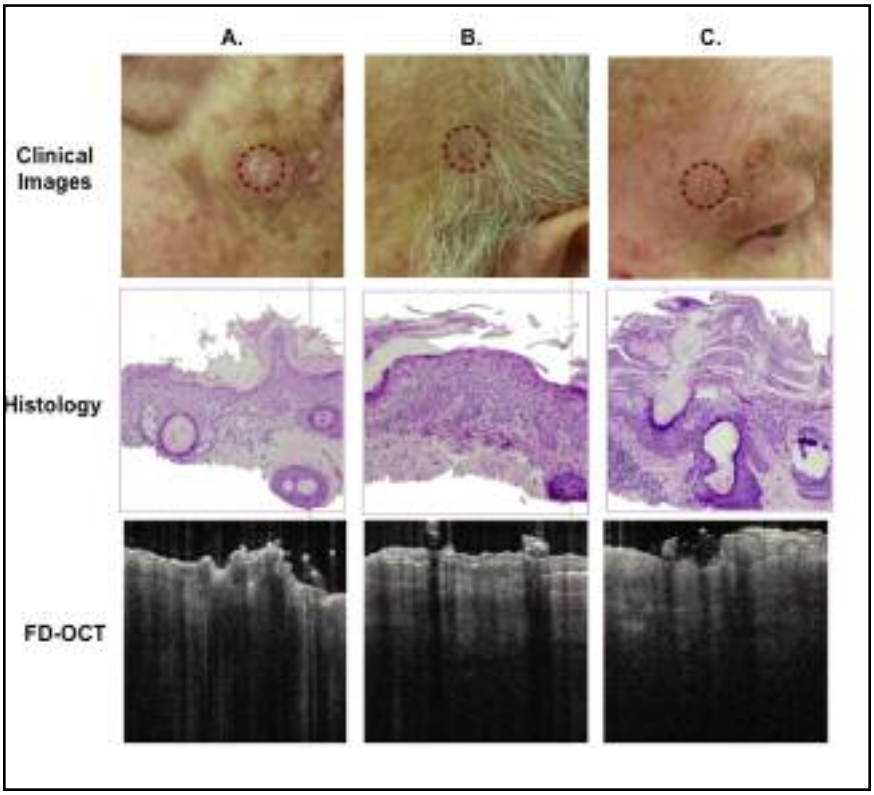


Figure 6. Comparison of clinical, histologic, and noninvasive findings. Row 1 represents clinical images, while rows 2 and 3 show the corresponding histology and FD-OCT scans, respectively. Columns A and B illustrate AK, while column C illustrates an SCC *in situ*. Note that the extent of histologic epidermal involvement increases from left to right, as does the corresponding FD-OCT scan; clinical severity decreased from left to right.

RESULTS

Subject demographics and baseline data. A total of 30 Caucasian male subjects were enrolled in this 60-day study (Figure 2). Two subjects did not return for follow-up visits and thus were not included in the analysis. The average (range) age of the subjects was 76 (67–93) years. The mean time since first AK diagnosis was seven years. All subjects had a prior history of treatment for AK, with cryotherapy, tretinoin, and fluorouracil being the most common treatments (Table 1).

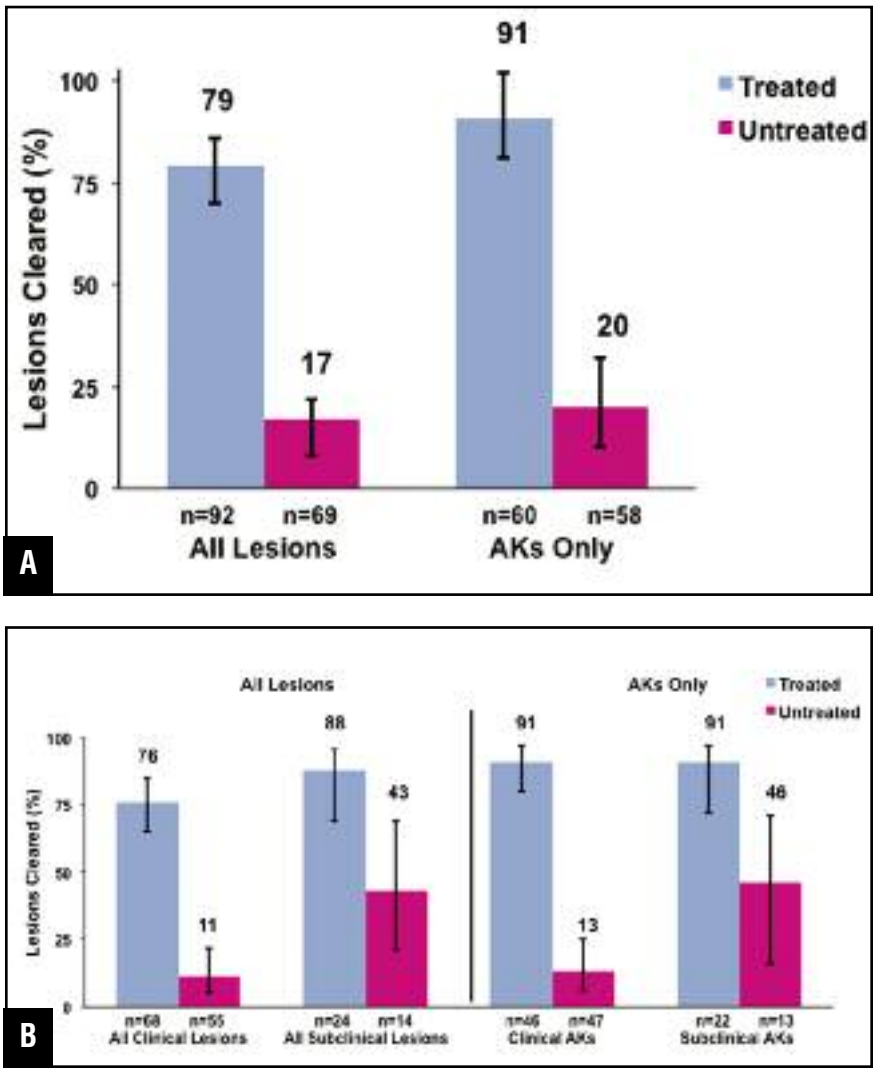
Correlation of biopsy-confirmed lesions with OCT. Histopathologic analysis was used to positively identify AK in biopsied samples (Figure 4B). At baseline, 28 of 28 (100%) of the clinically detected lesions were identified as AK, hypertrophic actinic keratosis (HAK), or SCC on biopsy. There were 21 AKs, four HAKs, and three SCC *in situ* (SCCIS) lesions. Within normal-appearing adjacent regions, 22 of 28 (79%) of the biopsied tissue samples were identified as AK or SCC (subclinical lesions). OCT images of these biopsies, examined in a blinded fashion, correctly identified 28 of 28 (100%; 95% CI, 88% 100%) of the clinical lesions and 16 of 22 (73%; 95% CI, 52–87%) of the subclinical lesions (Figure 5). These results provide evidence that OCT is more effective at discovering clinical lesions than subclinical lesions, although the confidence intervals for OCT almost overlap. Figure 6 shows three examples of an inverse relationship between clinical examination and histology, as compared with direct correlation between OCT examination and histology.

Correlation of OCT and clinically assessed baseline diagnoses. Clinical assessment did not always correlate with the results obtained by FD-OCT. Frequently, lesions were observed that were of mild clinical severity, while FD-OCT revealed them to be more advanced AKs or SCCs. Specifically, clinical assessment diagnosed 336 AKs (168 clinical and 168 perilesional, sun-exposed sites). Half of these suspected clinical (84/168) and subclinical (84/168) AKs were treated, and the other half of the clinical (84/168) and subclinical (84/168) AKs were left untreated. At baseline, all 336 suspected AKs, as identified by clinical assessment, were tested with the noninvasive OCT method. Diagnosis with the OCT technique showed that many of the suspected AKs were either normal skin or were more advanced lesions. Overall, OCT identified 123 of 168 (73%) clinically appearing AKs, and 38 of 168 (23%) perilesional-appearing AKs. Of the 84 treated clinical lesions, 68 of the lesions were identified as AK or more advanced lesions based on OCT, with 46 of the 68 positive lesions identified as AK. Of the 84 treated subclinical lesions, 24 of the lesions were identified as AK or more advanced lesions based on OCT, with 22 of the 24 positive lesions identified as AK. Of the 84 untreated clinical lesions, 55 of the lesions were identified as AK or more advanced lesions based on OCT, with 47 of the 55 positive lesions identified as AK. Of the 84 untreated subclinical lesions, 14 of the lesions were identified as AK or more advanced lesions based on OCT, with 13 of the 14 positive lesions identified as AK.

Effectiveness of ingenol mebutate treatment. In areas of the skin treated with ingenol mebutate gel 0.015%, OCT identified 92 lesions (sum of clinical and subclinical) at baseline, and 19 remaining lesions at Day 60, for a 79 percent (95% CI, 70–86%) lesion clearance rate (73 of 92 lesions cleared) (Figure 7A). When more advanced lesions such as HAKs, Bowen's disease, and SCCs were excluded from the analysis, 68 lesions were present at baseline and six lesions were present at Day 60, for a 91 percent (95% CI, 82–96%) lesion clearance rate (62 of 68 lesions cleared). In contrast, there was little change in the number of untreated lesions. There were 69 lesions in the untreated area at baseline, and 57 lesions in the area at Day 60, for a 17

percent (95% CI, 10–28%) clearance rate (12 of 69 lesions cleared). When more advanced lesions were excluded, there were 60 lesions in the untreated area at baseline and 48 lesions at Day 60, for a 20 percent (95% CI, 12–32%) clearance rate (12 of 60 lesions cleared). The differences between the proportions of lesions cleared with and without treatment are stark for both the all-lesions group and the AK-only subset.

Lesion clearance data were further analyzed to separately determine the effect of ingenol mebutate on clinical and subclinical lesions. Clinical lesion clearance rates were 76 percent (95% CI, 65–85%) (52 of 68 lesions cleared) and 91 percent (95% CI, 80–97%) (42 of 46 lesions cleared) for all clinical lesions and only clinical AKs, respectively, versus 11 percent (95% CI, 5–22%) (6 of 55 lesions cleared) and 13 percent (95% CI, 6–25%) (6 of 47 lesions cleared) for untreated lesions. For subclinical lesions, ingenol mebutate treatment cleared 88 percent (95% CI, 69–96%) (21 of 24 lesions cleared) of total subclinical lesions and 91 percent (95% CI, 72–97%) (20 of 22 lesions cleared) of subclinical AKs (Figure 7B). In contrast, 43 percent (95% CI, 21–67%) (6 of 14 lesions cleared) of subclinical lesions and 46 percent (95% CI, 23–71%) (6 of 13 lesions cleared) of subclinical AKs cleared with no treatment (Figure 7B). Taken together, these results show the effectiveness of ingenol mebutate in the treatment of AK, especially in clearing clinically typical lesions.



Figures 7A and 7B. Effect of ingenol mebutate on lesion clearance. Lesions were either treated with ingenol mebutate gel 0.015% for three consecutive days or left untreated. Subjects returned after 60 days, and the remaining lesions were identified using OCT. The number of lesions at baseline (n) is shown for each condition. Data are presented as the percentage of lesions at baseline that were clear at Day 60. Clinical and subclinical lesion data are combined in (A) and presented separately in (B).

LSR severity and lesion clearance rates. To assess the relationship between LSR severity and treatment effect, the authors compared lesion clearance among subjects with mild (n=9), moderate (n=12), or severe (n=7) LSRs at Day 3. Lesion clearance rates assessed by OCT were 77 percent (23 of 30), 79 percent (27 of 34), and 82 percent (23 of 28) in subjects with mild, moderate, and severe LSRs, respectively (Figure 8A); these differences were not significant (Chi-squared test p=0.8758). Similar lesion clearance rates were reported by both OCT and clinical assessment (Figure 8A). Excluding more advanced lesions from this analysis resulted in a more pronounced treatment effect, but it did not result in significant differences among LSR groups (Chi-squared test p=0.6228) (Figure 8B). The Chi-squared test results showed that there was no evidence to suggest that a subject's LSR severity was related to the treatment efficacy for both the all-lesions results and the AK-only subset.

DISCUSSION

AK presents special challenges to clinicians in the detection, monitoring, and treatment of lesions. Visual inspection typically cannot detect early changes present in subclinical lesions, and it is impractical for patients to undergo repeated, invasive biopsies of lesions that are clinically suspicious. Clinical diagnosis has been estimated to have a positive predictive value of approximately 75 to 95 percent when compared with conventional histopathologic analysis.[17–19] Biopsy sampling is not reasonable for the investigation of AK field cancerization, and clinical observation does not provide enough information about the overall field of damage. In the authors' study, they observed lesions that were of mild clinical severity, but which were

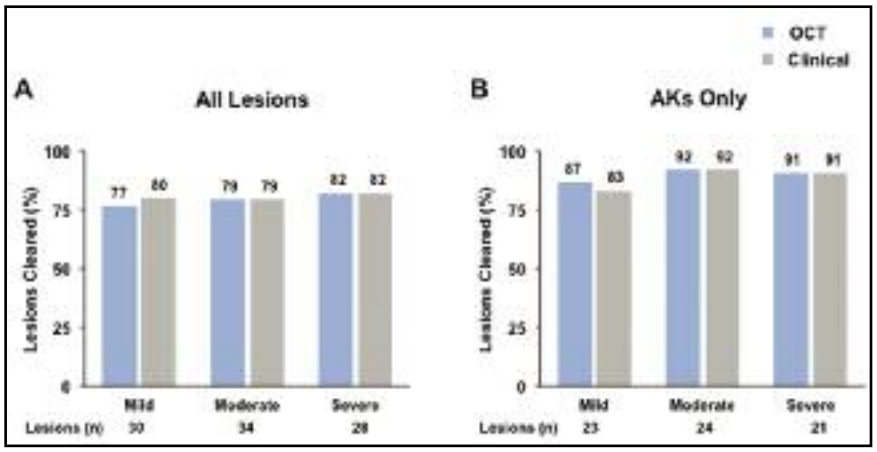


Figure 8. Comparison of OCT with clinical assessment in the evaluation of lesion clearance. Responses were grouped according to whether the subject's Day 3 LSRs were mild (n=9), moderate (n=12), or severe (n=7). The number of lesions present at baseline is shown under each condition. Data are presented as the percentage of lesions at baseline that were cleared at Day 60 (A). A separate analysis was performed with exclusion of HAK, Bowen's, or SCC lesions from the analysis (B).

later found to be more advanced when assessed by OCT. Subsequent correlation of the OCT determination with histology indicated that OCT could be a useful supplement to clinical assessment when biopsy is not an option. Together with clinical assessment, OCT can provide a fuller picture of the extent of the disease.

Recent studies have shown the utility of OCT as a noninvasive imaging technique that can be used to diagnose and monitor AKs. A study of 112 subjects whose AKs were evaluated using FD-OCT found that AK could be distinguished from nondiseased skin with 86 percent sensitivity and 83 percent specificity.[20] Significantly, intra-subject variability was much lower than inter-subject variability. Thus, repeated imaging of the same lesion in the same patient over time offers the possibility of further improvements in OCT performance. The authors' finding that FD-OCT could correctly identify 100 percent of clinical lesions on reference skin sites in 28 subjects is consistent with these high, reported levels of sensitivity. However, of the 168 clinical lesions (treated and untreated) identified as AKs on clinical examination, 73 percent were found to be AKs or more advanced lesions upon OCT. This discrepancy is likely due to the difference of recruiting one clinically biopsied lesion versus a cluster of three suspected clinical lesions. The authors believe that the inflammatory, irritant components of the damaged field could affect the clinical appearance of nearby benign lesions.

Key limitations of the authors' study were that their study population consisted of only 28 subjects, all of whom represented very similar demographics, and the use of a single, blinded dermatologist to read the OCT scans.

Successful identification of biopsy-confirmed subclinical lesions with OCT, in 16 of 22 cases (73%) in the authors' study, was an important finding. Identification of subclinical lesions by FD-OCT was recently reported by Themstrup et al, who used this technology to monitor lesions following methyl aminolevulinate photodynamic therapy (MAL-PDT).[14] At the three-month follow-up, OCT identified 29 percent more lesions (2 of 7) than did clinical examination alone (0 of 7). The two identified lesions did not become clinically apparent until four and nine months later, respectively, resulting in a significant delay in their treatment. Such findings suggest that the accuracy of FD-OCT might allow for the earlier detection of subclinical lesions and evaluation for treatment.

Consistent with observations made in the two pivotal clinical trials of ingenol mebutate,[21] the authors' study showed that a three-day treatment regimen with ingenol mebutate gel 0.015% cleared approximately 80 percent of clinical lesions. The clearance rate was above 90 percent when more advanced lesions, for which ingenol mebutate treatment is not indicated, were excluded. Importantly, ingenol mebutate also cleared 88 percent of subclinical lesions versus a clearance rate of 43 percent for untreated lesions. These results further define the concept of field cancerization in the context of AK and demonstrate the effectiveness of ingenol mebutate as a treatment approach.

With further validation, noninvasive imaging technology such as OCT is likely to become more frequently used in dermatologic practices. Compared with conventional biopsy, OCT imaging offers rapid imaging with the potential to not only diagnose clinical AKs but also detect subclinical AKs. Furthermore, the ability to monitor AK response to field-directed therapy in a noninvasive manner should be of broad interest to dermatologists. This may reduce the need for invasive biopsies and enable better lesion monitoring throughout treatment. It is critical, when treating clinical and subclinical AKs across an entire sun-exposed field, to be able to assess treatment response.

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REFERENCES

1. Rigel DS, Stein Gold LF. The importance of early diagnosis and treatment of actinic keratosis. *J Am Acad Dermatol.* 2013;68(1 Suppl 1):S20–S27.
2. Elsner P, Blome O, Diepgen TL. UV-induced occupational skin cancer: possibilities of secondary individual prevention in the "Dermatologist's Procedure." *J Dtsch Dermatol Ges.* 2013;11:625–630.
3. Berman B, Cockerell CJ. Pathobiology of actinic keratosis:

ultraviolet-dependent keratinocyte proliferation. *J Am Acad Dermatol.* 2013;68(1 Suppl 1):S10–S19.
4. Padilla RS, Sebastian S, Jiang Z, et al. Gene expression patterns of normal human skin, actinic keratosis, and squamous cell carcinoma: a spectrum of disease progression. *Arch Dermatol.* 2010;146:288–293.
5. Schwartz RA. The actinic keratosis. A perspective and update. *Dermatol Surg.* 1997;23:1009–1019.
6. Cockerell CJ, Wharton JR. New histopathological classification of actinic keratosis (incipient intraepidermal squamous cell carcinoma). *J Drugs Dermatol.* 2005;4:462–467.
7. Dodson JM, DeSpain J, Hewett JE, Clark DP. Malignant potential of actinic keratoses and the controversy over treatment. A patient-oriented perspective. *Arch Dermatol.* 1991;127:1029–1031.
8. Slaughter DP, Southwick HW, Smejkal W. Field cancerization in oral stratified squamous epithelium; clinical implications of multicentric origin. *Cancer.* 1953;6(5):963–968.
9. Goldenberg G, Perl M. Actinic keratosis: update on field therapy. *J Clin Aesthet Dermatol.* 2014;7:28–31.
10. Malvehy J. A new vision of actinic keratosis beyond visible clinical lesions. *J Eur Acad Dermatol Venereol.* 2015;29(Suppl 1):3–8.
11. Gambichler T, Jaedicke V, Terras S. Optical coherence tomography in dermatology: technical and clinical aspects. *Arch Dermatol Res.* 2011;303:457–473.
12. Picato (ingenol mebutate) gel 0.015%, 0.05% [package insert]. Parsippany, NJ: Leo Pharma Inc; 2015.
13. Maier T, Cekvic D, Ruzicka T, et al. Treatment monitoring of topical ingenol mebutate in actinic keratoses with the combination of optical coherence tomography and reflectance confocal microscopy: a case series. *Br J Dermatol.* 2015;172:816–818.
14. Themstrup L, Banzhaf CA, Mogensen M, Jemec GB. Optical coherence tomography imaging of non-melanoma skin cancer undergoing photodynamic therapy reveals subclinical residual lesions. *Photodiagnosis Photodyn Ther.* 2014;11:7–12.
15. Barton JK, Gossage KW, Xu W, et al. Investigating sun-damaged skin and actinic keratosis with optical coherence tomography: a pilot study. *Technol Cancer Res Treat.* 2003;2:525–535.
16. Brown LD, Cai TC, DasGupta A. Interval estimation for a binomial proportion. *Stat Sci.* 2001;16:101–133.
17. Venna SS, Lee D, Stadecker MJ, Rogers GS. Clinical recognition of actinic keratoses in a high-risk population: how good are we? *Arch Dermatol.* 2005;141:507–509.
18. Thompson SC, Jolley D, Marks R. Reduction of solar keratoses by regular sunscreen use. *N Engl J Med.* 1993;329:1147–1151.
19. Ponsford MW, Goodman G, Marks R. The prevalence and accuracy of diagnosis of non-melanotic skin cancer in Victoria. *Australas J Dermatol.* 1983;24:79–82.
20. Korde VR, Bonnema GT, Xu W, et al. Using optical coherence tomography to evaluate skin sun damage and precancer. *Lasers Surg Med.* 2007;39:687–695.
21. Lebwohl M, Swanson N, Anderson LL, et al. Ingenol mebutate gel for actinic keratosis. *N Engl J Med.* 2012;366:1010–1019.